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Tryptophan Degradation in Patients Infected by Human Immunodeficiency Virus

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Summary: Tryptophan and kynurenine were measured retrospectively in sera of 11 male patients with advanced human immunodeficiency virus (HIV) infection (Walter Reed stages 4 and 6). Tryptophan levels are significantly reduced to less than 50% in patients with HIV infection and kynurenine levels significantly elevated when compared to sex and age matched controls. The decrease of tryptophan levels might contribute to neurologic symptoms often associated with HIV infection. Since interferon-γ induces degradation of tryptophan via the kynurenine pathway, the present results may be consistent with enhanced endogenous production of interferon-γ in advanced HIV infection.

Tryptophan-Abbau bei HIV-infizierten Patienten

Zusammenfassung: Tryptophan und Kynurenin wurden retrospektiv im Serum von 11 Patienten (alle männlich) mit fortgeschrittener HIV-Infektion (Walter-Reed-Stadium 4 bzw. 6) bestimmt. Tryptophan war verglichen mit Gesunden gleichen Geschlechts und Alters signifikant erniedrigt und Kynurenin erhöht. Die beobachteten niedrigen Tryptophanwerte könnten zu den neurologischen Symptomen beitragen, die häufig bei HIV-Infizierten auftreten. Da Interferon-γ den Tryptophanabbau über Kynurenin als Zwischenprodukt induziert, sind unsere Ergebnisse mit Berichten über vermehrte Produktion von Interferon-γ bei Patienten mit fortgeschrittener HIV-Infektion vereinbar.

Key words: Degradation of tryptophan, kynurenine, neopterin, infection by human immunodeficiency virus, endogenous interferon-γ.

A great deal of evidence has accumulated within the last few years demonstrating that early steps of T-cell macrophage interaction are activated in patients with AIDS. This observation is supported by several findings among them highly elevated levels of neopterin, a product of interferon-γ-activated macrophages, in urine[1], serum[2,3] and cerebrospinal fluid[4] of patients with AIDS. In addition, interferon-γ was detected in sera of AIDS patients[5]. Interferon-γ is known to induce tryptophan degradation via the kynurenine pathway. In vitro[6] as well as in vivo[7,8] the degradation of tryptophan correlates to increased synthesis of neopterin. Thus, degradation of trypotphan via the kynurenine pathway can be expected to occur in patients

Abbreviations: HIV, human immunodeficiency virus; AIDS, acquired immunodeficiency syndrome; ARC, AIDS-related complex; WR, Walter Reed Staging classification; HPLC, high-performance liquid chromatography.

EXHIBIT B

with advanced HIV infection. We have examined retrospectively tryptophan and kynurenine concentrations in sera of patients infected by HIV and compared to neopterin concentrations. We have found significant decrease of tryptophan levels in patients which, by its impact on brain serotonin metabolism, might contribute to neurologic symptoms often associated with HIV infection.

Patients and Methods

Eleven male patients, aged 39 ± 10 years, were classified to stages WR4 (n = 5) and WR6 (n = 6) according to the Walter Reed staging classification[9]. The control group consisted of eleven male blood donors, aged 40 ± 12 years. Kynurenine, neopterin and creatinine were analysed in sera by HPLC[10]. Kynurenine was detected by fluorescence (excitation 353 nm, total emission) and ultraviolett absorption (260 nm) with a detection limit of 1 μmol/l, neopterin and creatinine were determined as described[10]. The sum of free and protein-associated, but not covalently bound tryptophan, was measured in serum diluted with a ten-fold volume of aqueous 0.9% sodium chloride by HPLC as described[6]. P-values for statistical significance were computed by Student's t-test and confirmed by Mann Whitney U-test. All figures shown are means ± standard deviations.

Results

Comparing patients to controls, serum levels of tryptophan are decreased to less than 50% and serum levels of kynurenine are elevated. Thus, the ratio of tryptophan per kynurenine drops by a factor of three (see table). Mean levels of neopterin in serum are eight times higher in patients than in controls. All differences are highly significant (see table). The degree of significance remains unchanged when the levels are related to the concomitantly measured creatinine, which is lower in the patients with HIV-related disease (70.9 ± 11.7 μmol/l) than in the controls (101.7 ± 17.6 μmol/l).
The figure displays the individual concentrations of tryptophan, kynurenine and neopterin. Whereas kynurenine levels show some overlap, all tryptophan levels are lower and all neopterin levels are higher in patients compared to controls.

Discussion

The increase of kynurenine and the decrease of tryptophan, which remained significant upon relating levels to creatinine, provide evidence that the lower tryptophan levels of the patients infected by HIV originate from tryptophan degradation via the kynurenine pathway rather than from secondary phenomena such as altered utilization of dietary tryptophan. The finding of increased levels of arginine and glutamate[11] underlines that the depletion of tryptophan reflects a specific phenomenon rather than a general decrease of amino-acid levels in HIV-infected patients. Since interferon-γ induces tryptophan degradation via the kynurenine pathway[6–8], the data presented in this work may be consistent with enhanced endogenous interferon-γ production in advanced infection by HIV[5]. The extent of the degradation of tryptophan and of raised levels of neopterin found in HIV-infected patients correspond to results obtained after administration of interferon-γ to cancer patients[8].

The observed catabolism of tryptophan might contribute to neurologic symptoms often accompanying HIV infection by withdrawal of the tryptophan required for serotonin synthesis

Table. Tryptophan, kynurenine and neopterin concentrations in sera of AIDS and ARC patients compared to blood donors (figures are means ± standard deviation).

	Patients	Controls	P*
Numbers of subjects	11	11	
Age	39 ± 10	40 ± 12	
Tryptophan [μmol/l]	44.8 ± 8.4	91.0 ± 22.0	< 0.0001
Kynurenine [μmol/l]	3.53 ± 0.89	2.31 ± 0.77	0.003
Tryptophan/kynurenine	13.4 ± 3.7	42.5 ± 13.7	< 0.0001
Neopterin [nmol/l]	39.1 ± 17.0	4.5 ± 1.5	< 0.0001

* Student's t-test.

KT → .008 ⇒ .025

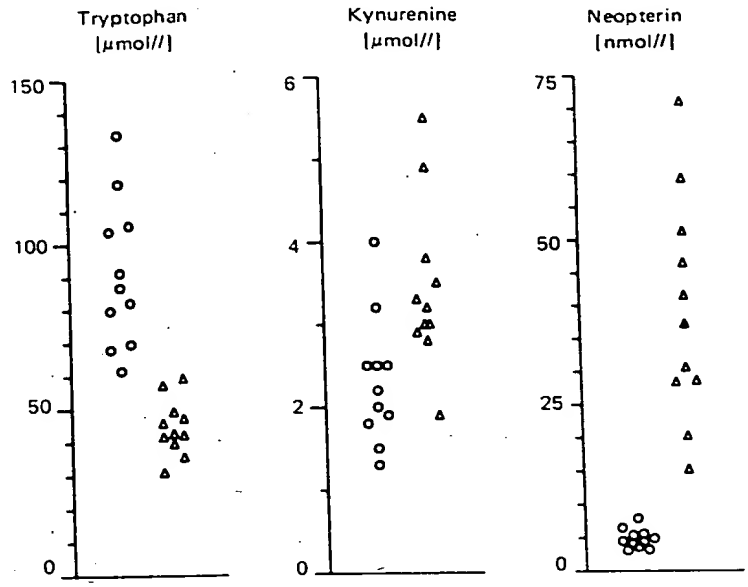

Figure. Serum concentrations of tryptophan, kynurenine and neopterin.
○ Blood donors; △ patients infected by HIV.

The concentrations of serotonin in brain physiologically depend on plasma tryptophan levels[12], which we show here to be substantially decreased in advanced infections by HIV. Further, several findings render plausible that in addition to the observed decrease of tryptophan levels in peripheral blood, tryptophan will also be degraded in the central nervous system. Immunologic reactions against HIV in the brain have been demonstrated in AIDS patients with neurologic symptoms[13]. It is to be anticipated that this reactions might be accompanied by increased endogeneous production of interferon-γ in the brain. This assumption is supported by extremely high neopterin levels observed in the cerebrospinal fluid of AIDS patients[4]. Thus, in addition to the impaired supply of tryptophan from the periphery, further decrease of tryptophan likely occurs in the brain in case of intracerebral viral infection leading to a severely impaired brain serotonin metabolism, which is associated with a variety of neurologic symptoms[14–16]. We suggest, therefore, that the disorder of the brain serotonin metabolism due to decrease of tryptophan levels should be considered as a potential source of neurologic symptoms in patients infected by HIV. The proposed mechanism can easily explain several features of HIV-related neurologic symptoms, for example the striking coincidence of acute encephalopathy with seroconversion for HIV[17]. To what extent concomitant infections contribute in addition to HIV to the tryptophan degradation observed here remains to be elucidated. In any event, our observations might provide a rationale for the therapy of neurologic symptoms in AIDS.

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